PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Coffee, Including Caffeinated and Decaffeinated Coffee, and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis
AUTHORS	Kennedy, Oliver; Roderick, Paul; Buchanan, Ryan; Fallowfield, Jonathan; Hayes, Peter; Parkes, Julie

VERSION 1 - REVIEW

REVIEWER	Guruprasad P.Aithal NIHR Nottingham Digestive Diseases Biomedical Research Unit
REVIEW RETURNED	27-Aug-2016

GENERAL COMMENTS	This is a met-analysis of the effect of coffee consumption on the risk of HCC. Authors demonstrate that coffee reduces the risk of HCC irrespective of underlying chronic liver disease or its aetiology. The analysis is robust and the demonstration of reduction in risk in association with decafinated driks add to the literature. Minor comments: 1) Page 3- Authors state that 'This is the first meta-analysis to calculate clinically relevant RRs of HCC..'- clarify what is clinically relevant? 2) Introduction: The sentence needs editing- Only a minority of patients present at a stage where they are eligible for potentially curative interventions (such as liver transplantation or partial liver resection), and the availability of such treatments is limited in areas most affected by HCC.- what is 'partial' liver resection? What proportion qualify for these treatment? Cite a reference. If not remove this sentence completely. 3) Authors refer to 'extra 2 cups' throughout the results, table and discussion. This needs clarification. What baseline cofee drinking should be assumed when refering to 'extra cup'? Is there a threshold beyond which 'extra cups' won't add any benefit?

REVIEWER	CHRISTINA BAMIA National and Kapodistrian University of Athens, Medical School, Department of Hygiene, Epidemiology and Medical Statistics
REVIEW RETURNED	30-Aug-2016

GENERAL COMMENTS	RE: “Caffeinated and Decaffeinated Coffee Consumption and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis” This meta-analysis evaluates the association of coffee intake with HCC risk. Additional analyses have been also undertaken on the

	association of caffeinated and decaffeinated coffee intake, as well as, on the modification of the indicated associations by chronic liver Disease (CLD) and other HCC risk factors. The authors concluded on an inverse association of both caffeinated and decaffeinated coffee with HCC risk, overall, as well as, among patients with pre-existing liver disease. The meta-analysis has been conducted appropriately and carefully. A quite extended Discussion, especially regarding potential mechanisms which may explain the indicated inverse associations is included in the manuscript. Some comments for the authors' consideration follow: 1. The apparent "protection" of coffee intake on HCC risk has been reported, especially in the recent years. The meta-analysis by Bravi and colleagues (2013) included all studies published up to September 2012. These authors estimated RR for HCC associated with various levels of coffee intake as well as for increments of 1 cup of coffee per day with results that agree with those presented in the current manuscript. The most recent meta-analysis (2016) was undertaken by Bravi and colleagues (some of the authors contributed also to the 2013 meta-analysis), and examined coffee in association to the risk of HCC using only cohort studies published apparently up to 2015 - results were similar to the 2013 and to the current meta-analysis but a detailed analysis on coffee in relation to CLD risk was additionally included in the 2016 meta-analysis. Moreover, in the context of the WCRF 2015 Continuous Updated Project (CUP), all studies reporting on coffee intake and liver cancer published up to June 2013 were included in a meticulous meta-analysis with a detailed dose-response evaluation. Notably, in that report the level of evidence regarding the inverse association of coffee intake and liver cancer was changed since the 2007 WCRF Second Expert Report and it was judged as "probable". The 2015 Report notes that: "There is strong evidence that drinking coffee is linked to a decreased risk of liver cancer" and that "Higher consumption of coffee probably protects against liver cancer". In May 2016 a Working Group of scientists were invited by the International Agency for Research on Cancer (IARC) to assess the carcinogenicity of drinking coffee (and other beverages). A summary of their overall evaluation, based on all published evidence up to May 2016 was published in Lancet Oncology (LO) stating that "…Inverse associations with coffee drinking were also observed in cohort and case-control studies of liver cancer in Asia, Europe, and North America. A meta-analysis of prospective cohort studies estimated that the risk of liver cancer decreases 15% for each 1 cup per day increment…" (refers to the Bravi et al, 2016 meta-analysis). In the light of the 2013 and 2016 meta-analyses as well as of the WCRF and IARC reports the authors may wish to expand on the "new" issues that their study add to the already published evidence. They may also consider commenting on the WCRF and IARC publications (which are not apparently cited in the current manuscript). 2. The authors of the current manuscript also state that this is the first meta-analysis to calculate a RR of HCC for decaffeinated coffee intake in relation to HCC risk. Their pooled estimated RR is, however based only on three cohort studies and one case-control on subjects with CLD. None of these studies individually reported a statistically significant association between HCC and decaffeinated coffee consumption but the pooled RR of HCC for increment of 2 c/day was 0.86 (95% CI 0.74-1.00) and borderline statistically significant. Most published studies have not distinguished between

	caffeinated and decaffeinated coffee intake, although it is legitimate to assume that the coffee consumed in these studies is in the vast majority mainly caffeinated. Based on these arguments it would seem premature to argue on a definite evaluation of the relative risk for HCC associated with decaffeinated coffee, which would allow drawing conclusions with epidemiological and public health relevance. Based on what has been stated in this comment some of the authors' conclusions such as "increased consumption of both caffeinated and decaffeinated coffee is associated with reduced risk of HCC, including in pre-existing liver disease" or, that the observed decreased risk of HCC associated with decaffeinated coffee intake "…has importance for developing coffee as a lifestyle intervention in CLD, as decaffeinated coffee might be more acceptable to those who do not drink coffee or who limit their coffee consumption because of caffeine related symptoms", may not be fully justifiable by the current findings. I would suggest that the authors down-weight their conclusions regarding decaffeinated coffee. 3. The authors have explored the modification of the inverse association between coffee and HCC by key risk factors, such as HBV/HCV, high body mass index (BMI), type-2 diabetes mellitus (T2DM), alcohol consumption and the presence of CLD including cirrhosis – they mainly focused on the latter risk factor. They also (as previous meta-analyses) found no evidence of effect modification by any of those but the respective analyses were based on a small number of studies. On the other hand, the power of these analyses is low and therefore definite conclusions cannot be drawn. The authors may wish to take these points into account when interpreting their findings especially with respect to previous CLD (yes/no). Only three studies have reported on previous CLD and, perhaps, statements such as the one in page 16, lines 3-7 are indicative of over-interpretation. 4. In relation to the investigation of modification of the association among people with risk factors for HCC, I would expect also smoking to be included as this is a reported risk factor for HCC and strongly correlated with coffee intake. 5. Regarding the Greenland and Longnecker method could the authors briefly mention when was it possible to be used with respect to the studies included? 6. I find the sentences "Where the number of exposed and non-exposed were not available to correct for covariance, we used variance-weighted least squares regression. We meta-analysed the differences between the stratified RRs to test for statistical significance" confusing with respect to the statistical methods appointed for these analyses. Perhaps the authors could be more explicit? 7. The authors have also performed an analysis on absolute risk reduction, using GRADE (table 3), according to which the evidence quality that coffee protects against HCC was "very low". I m concerned that this may be perceived by the readers as a contradiction to what has been seen so far (see comment 1) regarding coffee in association to HCC, including findings of the current manuscript. Therefore, I would suggest that the authors devote some space in explaining the apparent "contradiction". 8. The authors may consider reducing some of the text devoted to mechanisms which seems too long especially when current evidence is rather suggestive up to now.

REVIEWER	Akira Kuriyama Department of General Medicine, Kurashiki Central Hospital
REVIEW RETURNED	17-Oct-2016

GENERAL COMMENTS	Dr. Kennedy et al. examined the relationship between coffee consumption and the risk of hepatocellular carcinoma (HCC) using a meta-analytic method. Their study suggested an increased amount of coffee consumption, either caffeinated or decaffeinated, was associated with a reduced risk of HCC development. This study is quite rigorously conducted and here are my comments. Major: - I cannot see why the authors pooled the data into RR (risk ratio). I understand the rationale that "the low incidence of HCC, we considered ORs, RRs, HRs to be equivalent", but I feel somewhat awkward to see RR from case-control studies. Do the authors consider reporting OR (odds ratio) instead of RR? Did the interpretation of the results differ between OR and RR? - The authors mentioned the effect size from 7 excluded studies due to the lack of effect size reporting cannot be similar to the current findings from 16 studies. Cautions are still needed. Minor: - In the manuscript, the quality of the evidence according to GRADE was considered as "very low". Reflect this in the abstract. - Explain briefly the method of Greenland and Longnecker for readers. - The sentence in the Box "Full-text articles excluded" in Figure 1 is incomplete. - Excuse me if I am wrong. I did not see the pooled outcome of unadjusted RR. Was it different from that of adjusted RR?

REVIEWER	Sabrina Mai Nielsen Musculoskeletal Statistics Unit, The Parker Institute, Copenhagen University Hospital, Bispebjerg & Frederiksberg, Copenhagen, Denmark.
REVIEW RETURNED	30-Nov-2016

GENERAL COMMENTS	The systematic review investigates the risk of hepatocellular carcinoma from caffeinated and decaffeinated coffee, respectively. From the title I got the impression that the two coffee types would be compared, however, this was not the case - most studies did not distinguish between caffeinated and decaffeinated coffee, and the majority of their analyses are based on ’coffee’ as variable. However, they do present results separate for each coffee type, but these are based on only four studies. Therefore, I suggest that the title should be modified. I have reviewed this manuscript with a particular emphasis on the

	statistical methods and analyses used. The authors are commended for: - Well-described study selection, data-extraction - Also providing estimates separate for each study type (cohort vs. case-control), and separate for study quality score (6 or above vs. below 6) - Assessing the magnitude and direction of adjustment for publication bias Overall, it might be a caveat whether there was a pre-specified protocol. The authors do not report to have published or registered the protocol online, such as on PROSPERO, and it is therefore unclear if the protocol in the supplementary information is the original version from before conducting the search, or a corrected version according to study findings. This is potentially critical to BMJ Open. They extracted the most rigorously adjusted effect sizes, i.e. those adjusted for most factors. They consider ORs, RRs and HRs to be equivalent due to low incidence of HCC, referred to as RRs, which is probably a reasonable assumption. Their primary analysis is based on a random-effects, dose-response meta-analysis, and they assessed whether the dose-response was non-linear by a cubic spline meta-analysis. The rationale for using the exposure, 'extra two cups/day' should be mentioned in the text. Secondary, they tested for modification of the effect by exposures/risk factors, i.e. conducted stratified meta-regressions of the RRs, for a) stage of liver disease (presence vs. absence of chronic liver disease), b) viral hepatitis status (carriers of HBV/HCV vs. negative for both), c) BMI (highest vs. lowest BMI categories), d) T2DM (presence vs. absence), and e) alcohol consumption (highest vs. lowest categories). For their meta-regression analyses, they do not state which type of random effects model they are using, i.e. the method for estimating tau^2 (restricted maximum-likelihood, maximum likelihood, DerSimonian-Laird estimator. etc.). Please elaborate. Furthermore, for the stratified meta-regressions, it may be preferable to present a table with the results instead of only text, and including tau2, the p-value for the interaction etc., in order to provide an easier overview. Third, they investigated heterogeneity by meta-regression of publication year, length of follow-up (cohorts), percentage of alcohol abstainers, age, and gender, and examined the impact of individual studies in a sensitivity analysis. Potentially the authors could have considered using a proxy for person-years rather than length of follow-up.

	Other minor issues and comments: 1) They use the Newcastle Ottawa Scale for the risk of bias assessment. I suggest, that the authors discuss the impact of this tool on the assessment results compared to the new ROBINS-I tool. 2) The search strategy seems simple, and more relevant synonyms could maybe provide more eligible studies. Please discuss. 3) They excluded studies that "*did not report a dose-response or give sufficient information for calculation of a dose-response (i.e. this requires estimates for more than two exposure levels*" and do not state if they contacted authors in order to try to retrieve missing data. If only a few were missing, contacting authors may have been appropriate. Please discuss. 4) Their reference to the Cochrane handbook (heterogeneity), [22], should be changed into the primary references (i.e., *Higgins, BMJ 2003;327:557*) instead or the specific section/chapter. 5) In the "Article summary", the authors state "*This is the first meta-analysis to calculate clinically relevant RRs of HCC for 1-5 cups of coffee per day*", however, the criteria used for deeming the effect clinical relevant is not stated in the text, and 'clinically relevant' is not mentioned further in the text. Please discuss. 6) No references are provided for the R packages (citation info is available online). Please include. 7) The cubic spline dose-response meta-analysis is several places called 'cubic spine (…)'. Please remember 'L'. 8) The objectives are not stated in the main text. Furthermore, the objectives in the abstract and in the protocol are slightly ambiguous – you could get the impression, that caffeinated coffee will be compared to decaffeinated coffee. Please make it more clear to the reader.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Guruprasad P. Aithal

"This is a met-analysis of the effect of coffee consumption on the risk of HCC. Authors demonstrate that coffee reduces the risk of HCC irrespective of underlying chronic liver disease or its aetiology. The analysis is robust and the demonstration of reduction in risk in association with decafinated driks add to the literature."

We thank Reviewer 1 for these comments about our work.

"Minor comments:

1) Page 3- Authors state that 'This is the first meta-analysis to calculate clinically relevant RRs of

HCC..'- clarify what is clinically relevant?"

The term “clinically relevant” is used once in the first sentence of the article summary and not mentioned again in the text. To improve the clarity of that sentence, we have deleted “clinically relevant” and stated instead that our findings “may be useful in the design of a coffee based intervention for evaluation in a clinical trial”.

"2) Introduction: The sentence needs editing- Only a minority of patients present at a stage where they are eligible for potentially curative interventions (such as liver transplantation or partial liver resection), and the availability of such treatments is limited in areas most affected by HCC.- what is 'partial' liver resection? What proportion qualify for these treatment? Cite a reference. If not remove this sentence completely."

As suggested, we have added a reference and rewritten this passage as: “Only 10%-37% of patients diagnosed with HCC are eligible for potentially curative tumour resection (partial hepatectomy). Thus, prognosis remains poor with a 5-year overall survival rate of 18%” (references not shown).

"3) Authors refer to 'extra 2 cups' throughout the results, table and discussion. This needs clarification. What baseline cofee drinking should be assumed when refering to 'extra cup'? Is there a threshold beyond which 'extra cups' won't add any benefit?"

We agree with Reviewer 1 that there likely exists an upper limit beyond which our estimated RRs for an extra two cups of coffee per day will not apply. However, the upper limit was not apparent from the individual studies or from our test for non-linearity of the dose-response. This was likely because of a lack of data at high levels of consumption. Thus, we have amended the discussion section to highlight this limitation and uncertainty in our results, making reference to figure 3, which demonstrates rapidly increasing confidence intervals as consumption increases above 4 cups of coffee per day compared to none.

Reviewer 2: Christina Bamia

"This meta-analysis evaluates the association of coffee intake with HCC risk. Additional analyses have been also undertaken on the association of caffeinated and decaffeinated coffee intake, as well as, on the modification of the indicated associations by chronic liver Disease (CLD) and other HCC risk factors. The authors concluded on an inverse association of both caffeinated and decaffeinated coffee with HCC risk, overall, as well as, among patients with pre-existing liver disease. The meta-analysis has been conducted appropriately and carefully. A quite extended Discussion, especially regarding potential mechanisms which may explain the indicated inverse associations is included in the manuscript."

We thank Reviewer 2 for these comments.

"1. The apparent “protection” of coffee intake on HCC risk has been reported, especially in the recent years. The meta-analysis by Bravi and colleagues (2013) included all studies published up to September 2012. These authors estimated RR for HCC associated with various levels of coffee intake as well as for increments of 1 cup of coffee per day with results that agree with those presented in the current manuscript. The most recent meta-analysis (2016) was undertaken by Bravi and colleagues (some of the authors contributed also to the 2013 meta-analysis), and examined coffee in association to the risk of HCC using only cohort studies published apparently up to 2015 - results were similar to the 2013 and to the current meta-analysis but a detailed analysis on coffee in relation to CLD risk was additionally included in the 2016 meta-analysis. Moreover, in the context of the WCRF 2015 Continuous Updated Project (CUP), all studies reporting on coffee intake and liver cancer published

up to June 2013 were included in a meticulous meta-analysis with a detailed dose-response evaluation. Notably, in that report the level of evidence regarding the inverse association of coffee intake and liver cancer was changed since the 2007 WCRF Second Expert Report and it was judged as “probable”. The 2015 Report notes that: “There is strong evidence that drinking coffee is linked to a decreased risk of liver cancer” and that “Higher consumption of coffee probably protects against liver cancer”. In May 2016 a Working Group of scientists were invited by the International Agency for Research on Cancer (IARC) to assess the carcinogenicity of drinking coffee (and other beverages). A summary of their overall evaluation, based on all published evidence up to May 2016 was published in Lancet Oncology (LO) stating that “…Inverse associations with coffee drinking were also observed in cohort and case-control studies of liver cancer in Asia, Europe, and North America. A meta-analysis of prospective cohort studies estimated that the risk of liver cancer decreases 15% for each 1 cup per day increment…” (refers to the Bravi et al, 2016 meta-analysis). In the light of the 2013 and 2016 meta-analyses as well as of the WCRF and IARC reports the authors may wish to expand on the “new” issues that their study add to the already published evidence. They may also consider commenting on the WCRF and IARC publications (which are not apparently cited in the current manuscript)."

We thank Reviewer 2 for the useful information provided. We have cited the WCRF and IARC reports in the introduction.

As we have summarised in our manuscript, all the evidence supporting the effect of coffee for preventing HCC is observational. Accordingly, there is now a need to undertake randomised trials to establish a more robust evidence base. However, designing a suitable coffee based intervention is challenging, particularly because “coffee” is poorly defined (e.g. caffeinated vs. decaffeinated, boiled vs. filtered etc.) and effect modification by varied causes/risk factors for HCC is incompletely understood. The observational literature contains important information for understanding and overcoming those challenges. We explain below how our study has summarised this information at a meta-analytic level for the first time.

Firstly, we have calculated RRs of HCC for both caffeinated and decaffeinated coffee. We have shown that caffeinated coffee is more convincingly associated with a reduced risk of HCC but that there is also a weaker association with decaffeinated coffee (RR 0.86, 95% CI 0.74-1.00). Ours is the first meta-analysis to quantify these associations.

Second, Bravi et al. 2016[1] and the WCRF report only provide limited dose-response data, specifically a RR of HCC for a one cup per day increase from linear analyses. However, for the reasons explained above by Prof Aithal, it is important to investigate whether a threshold exists where increasing consumption does not provide further benefit. Using a model that allows for non-linearity of the dose-response, we provide numerical RRs of HCC for 1-4 cups per day compared to none and a graphical representation of the RRs for 1-5 cups per day. We show that there is a good response to increasing coffee consumption up to at least 4 cups per day but that the levels of uncertainty increase rapidly thereafter. This is important given that many people who will be targeted by a coffee based intervention will likely be pre-existing coffee drinkers.

Third, the fact coffee has been shown to protect against cirrhosis in previous studies clouds the reported inverse association between coffee and HCC. This is because cirrhosis is by far the biggest risk factor for HCC. Thus, if the protective effect against HCC acts solely through the prevention of cirrhosis, the protection against HCC would be uncertain if cirrhosis was already present. This requires clarification because an intervention will be targeted at people most at risk from HCC who are likely to have cirrhosis. Our analysis provides new insight by calculating RRs of HCC in patients with and without CLD, including cirrhosis, at baseline.

Fourth, protective mechanisms of action of coffee specific to particular aetiologies of CLD/HCC have been suggested in the literature. For example, coffee has been suggested to prevent liver disease by reducing the incidence or severity of diabetes or by interfering with replication of hepatitis B and C virus (see the manuscript for references). If the effect of coffee varies with aetiology, this needs to be taken into account when targeting an intervention. We have performed for the first time a meta-analysis of data from participants with and without all the major risk factors: alcohol, high BMI/diabetes, viral hepatitis and smoking (we have added the smoking data in response to Dr Bamia's fourth comment below). To reduce the influence of between-study effects, we compared participants from the same studies with and without the risk factors. We show that there is no detectable effect modification according to aetiology/risk factors.

Finally, we have performed a robust assessment of publication bias by calculating a common exposure category for each study before applying Egger's regression and by performing a trim-and-fill analysis. For the first time, we have detected potential publication bias in the estimated association.

We have amended the introduction section to make the new aspects of our work clearer.

"2. The authors of the current manuscript also state that this is the first meta-analysis to calculate a RR of HCC for decaffeinated coffee intake in relation to HCC risk. Their pooled estimated RR is, however based only on three cohort studies and one case-control on subjects with CLD. None of these studies individually reported a statistically significant association between HCC and decaffeinated coffee consumption but the pooled RR of HCC for increment of 2 c/day was 0.86 (95% CI 0.74-1.00) and borderline statistically significant. Most published studies have not distinguished between caffeinated and decaffeinated coffee intake, although it is legitimate to assume that the coffee consumed in these studies is in the vast majority mainly caffeinated. Based on these arguments it would seem premature to argue on a definite evaluation of the relative risk for HCC associated with decaffeinated coffee, which would allow drawing conclusions with epidemiological and public health relevance. Based on what has been stated in this comment some of the authors' conclusions such as "increased consumption of both caffeinated and decaffeinated coffee is associated with reduced risk of HCC, including in pre-existing liver disease" or, that the observed decreased risk of HCC associated with decaffeinated coffee intake "…has importance for developing coffee as a lifestyle intervention in CLD, as decaffeinated coffee might be more acceptable to those who do not drink coffee or who limit their coffee consumption because of caffeine related symptoms", may not be fully justifiable by the current findings. I would suggest that the authors down-weight their conclusions regarding decaffeinated coffee."

We have amended the abstract to state that "consumption of caffeinated and, to a lesser extent, decaffeinated coffee is associated with reduced risk of HCC...". In addition, we have amended the discussion to state that the association between HCC and decaffeinated coffee "may be important for developing coffee as a lifestyle intervention in CLD", and we have added a sentence immediately afterwards stating that "the benefits of decaffeinated coffee appear to be smaller and less certain than for caffeinated coffee". We note that guidance from the Cochrane Collaborative advises against descriptive terms such as "trend towards" and "borderline significant" hence we have avoided those and similar terms in the manuscript as far as possible.

"3. The authors have explored the modification of the inverse association between coffee and HCC by key risk factors, such as HBV/HCV, high body mass index (BMI), type-2 diabetes mellitus (T2DM), alcohol consumption and the presence of CLD including cirrhosis – they mainly focused on the latter risk factor. They also (as previous meta-analyses) found no evidence of effect modification by any of those but the respective analyses were based on a small number of studies. On the other hand, the power of these analyses is low and therefore definite conclusions cannot be drawn. The authors may wish to take these points into account when interpreting their findings especially with respect to

previous CLD (yes/no). Only three studies have reported on previous CLD and, perhaps, statements such as the one in page 16, lines 3-7 are indicative of over-interpretation."

We have amended the sentence at page 16, lines 3-7 to state that “We did not detect effect modification by baseline CLD and HCC aetiology, although our analysis was limited by the small number of studies that provided the necessary data for these analyses”. This statement fairly reflects the limitations of our results.

"4. In relation to the investigation of modification of the association among people with risk factors for HCC, I would expect also smoking to be included as this is a reported risk factor for HCC and strongly correlated with coffee intake."

We agree that smoking should also be investigated as a risk factor and we have updated the text and supplementary information accordingly.

"5. Regarding the Greenland and Longnecker method could the authors briefly mention when was it possible to be used with respect to the studies included?
6. I find the sentences “Where the number of exposed and non-exposed were not available to correct for covariance, we used variance-weighted least squares regression. We meta-analysed the differences between the stratified RRs to test for statistical significance” confusing with respect to the statistical methods appointed for these analyses. Perhaps the authors could be more explicit?"

We will address comments 5 and 6 together since they relate to similar issues. We have amended the methods section by adding a subheading entitled: “Effect modification by risk factors” and, under that subheading, referenced the studies which provided dose-response data or allowed use of the Greenland and Longnecker method. We have also indicated the corresponding studies used for the “Coffee and HCC” and “Caffeinated and decaffeinated coffee and HCC” analyses. Hence, it is now clear which studies provided dose-response data or allowed use of the Greenland and Longnecker method. We have also clarified the statistical test we used to assess the differences between the stratified RR and presented p-values in the supplementary information.

"7. The authors have also performed an analysis on absolute risk reduction, using GRADE (table 3), according to which the evidence quality that coffee protects against HCC was “very low”. I’m concerned that this may be perceived by the readers as a contradiction to what has been seen so far (see comment 1) regarding coffee in association to HCC, including findings of the current manuscript. Therefore, I would suggest that the authors devote some space in explaining the apparent “contradiction”."

We agree with Reviewer 2 that this point merits further attention in our manuscript. Thus, we have amended the discussion to explain that our study adds to the weight of evidence showing that coffee is protective against HCC. We have also explained how, at the same time, the quality of evidence under the GRADE criteria is still “very low” because of (i) the lack of randomised trials, (ii) the evidence of publication bias (which had not been detected previously) and (iii) indirectness because there being no accepted definition “coffee”, which comes in many formulations with varying chemical compositions. To highlight the principle that observational studies can be misleading, we already provide a reference and comment on a randomised controlled trial, which contradicted previous convincing evidence that had suggested vitamin D prevented colorectal cancer.

"8. The authors may consider reducing some of the text devoted to mechanisms which seems too long especially when current evidence is rather suggestive up to now."

We thank Reviewer 2 for this suggestion and have reduced the length of the discussion of the

mechanism of action accordingly.

Reviewer 3: Akira Kuriyama

"Dr. Kennedy et al. examined the relationship between coffee consumption and the risk of hepatocellular carcinoma (HCC) using a meta-analytic method. Their study suggested an increased amount of coffee consumption, either caffeinated or decaffeinated, was associated with a reduced risk of HCC development. This study is quite rigorously conducted and here are my comments."

We thank Reviewer 3 for these comments.

"Major:
- I cannot see why the authors pooled the data into RR (risk ratio). I understand the rationale that "the low incidence of HCC, we considered ORs, RRs, HRs to be equivalent", but I feel somewhat awkward to see RR from case-control studies. Do the authors consider reporting OR (odds ratio) instead of RR? Did the interpretation of the results differ between OR and RR?"

We state in the manuscript that “[g]iven the low incidence of HCC, we considered ORs, RRs, HRs to be equivalent, and for simplicity we use RR to refer to all three herein”. This is an approximation that is employed by most meta-analyses that include both cohort and case-control studies and is generally considered reasonable, as is acknowledged by Reviewer 4 below.

"- The authors mentioned the effect size from 7 excluded studies due to the lack of effect size reporting cannot be similar to the current findings from 16 studies. Cautions are still needed."

We assume that this comment refers to previous figure 1, which stated that seven studies were excluded for “No effect size reported or inappropriate study design…”. The studies excluded under this category were reviews/comments or did not report RRs for HCC according to coffee consumption, for example, because an exposure other than coffee was considered. They did not contain contradictory findings to our study and were frequently irrelevant to our research question. We have updated the text in the figure to make the reason for exclusion clearer.

"Minor:
- In the manuscript, the quality of the evidence according to GRADE was considered as "very low". Reflect this in the abstract."

We have amended the abstract to reflect the GRADE score, as suggested.

"- Explain briefly the method of Greenland and Longnecker for readers."

In addition to the reference already provided, we have added an explanatory sentence after the first mention of the Greenland and Longnecker method.

"- The sentence in the Box "Full-text articles excluded" in Figure 1 is incomplete."

We thank Reviewer 3 for bringing this to our attention and we have now amended the sentence in the box.

"- Excuse me if I am wrong. I did not see the pooled outcome of unadjusted RR. Was it different from that of adjusted RR?"

The unadjusted values are stated in the sentence above table 1 which reads: “Adjustment for

confounders had minimal effect, changing the pooled RR from 0.62 (95% CI 0.53-0.72) to 0.65 (95% CI 0.59-0.72)". We have added "(i.e. unadjusted)" after the corresponding RR to make this sentence easier to find.

Reviewer 4: Sabrina Mai Nielsen

"The systematic review investigates the risk of hepatocellular carcinoma from caffeinated and decaffeinated coffee, respectively. From the title I got the impression that the two coffee types would be compared, however, this was not the case - most studies did not distinguish between caffeinated and decaffeinated coffee, and the majority of their analyses are based on 'coffee' as variable. However, they do present results separate for each coffee type, but these are based on only four studies. Therefore, I suggest that the title should be modified."

We thank Reviewer 4 for these comments. The title of our work is Caffeinated and Decaffeinated Coffee Consumption and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis. Ours is the first and only meta-analysis to calculate separate RRs for decaffeinated and caffeinated coffee. Thus, it is beneficial to the potential reader that this is reflected by the title. The title is read in the context of the abstract, which states in some detail the methods used and the numbers of studies included in each of our analyses. Nonetheless, to reflect the fact that many of our analyses focus on "coffee" rather than specifically decaffeinated or caffeinated coffee, we have amended the title to "Coffee, Including Caffeinated and Decaffeinated Coffee, and the Risk of Hepatocellular Carcinoma: A Systematic Review and Dose-Response Meta-Analysis."

"I have reviewed this manuscript with a particular emphasis on the statistical methods and analyses used. The authors are commended for:
- Well-described study selection, data-extraction
- Also providing estimates separate for each study type (cohort vs. case-control), and separate for study quality
score (6 or above vs. below 6)
- Assessing the magnitude and direction of adjustment for publication bias"

We thank Reviewer 4 for these comments.

"Overall, it might be a caveat whether there was a pre-specified protocol. The authors do not report to have published or registered the protocol online, such as on PROSPERO, and it is therefore unclear if the protocol in the supplementary information is the original version from before conducting the search, or a corrected version according to study findings. This is potentially critical to BMJ Open."

The protocol was pre-specified but, similarly to many meta-analyses including in The BMJ and BMJ Open, was not pre-registered online. We have amended the methods section to make this clearer.

"They extracted the most rigorously adjusted effect sizes, i.e. those adjusted for most factors. They consider ORs, RRs and HRs to be equivalent due to low incidence of HCC, referred to as RRs, which is probably a reasonable assumption. Their primary analysis is based on a random-effects, dose-response meta-analysis, and they assessed whether the dose-response was non-linear by a cubic spline meta-analysis. The rationale for using the exposure, 'extra two cups/day' should be mentioned in the text."

We have amended the manuscript to state that the unit of an "extra two cups" per day was selected to represent a potential coffee based intervention, which could be used in clinical trials, and to maintain comparability with a previous meta-analysis.

"Secondary, they tested for modification of the effect by exposures/risk factors, i.e. conducted stratified metaregressions of the RRs, for a) stage of liver disease (presence vs. absence of chronic liver disease), b) viral hepatitis status (carriers of HBV/HCV vs. negative for both), c) BMI (highest vs. lowest BMI categories), d) T2DM (presence vs. absence), and e) alcohol consumption (highest vs. lowest categories). For their meta-regression analyses, they do not state which type of random effects model they are using, i.e. the method for estimating tau2 (restricted maximum likelihood, maximum likelihood, DerSimonian-Laird estimator. etc.). Please elaborate."

We have amended the manuscript to specify that we used the DerSimonian-Laird method.

"Furthermore, for the stratified meta-regressions, it may be preferable to present a table with the results instead of only text, and including tau2, the p-value for the interaction etc., in order to provide an easier overview."

The results referred to by Reviewer 4 are in the supplementary information. We have presented the data in the text (i.e. rather than as a table) to facilitate explanation of important differences in each analysis (e.g. different cut-offs for BMI or infection with HCB and/or HBV). The Uniform Requirements for Manuscripts Submitted to Biomedical Journals (ICMJE), which is referred to in the BMJ Open submission guidelines, states that studies should present “results in logical sequence in the text, tables, and figures…Do not repeat all the data in the tables or figures in the text.” Thus, for conformity with this guidance, we have not repeated the data in a table. However, we have added p-values and tau2, as suggested.

"Third, they investigated heterogeneity by meta-regression of publication year, length of follow-up (cohorts), percentage of alcohol abstainers, age, and gender, and examined the impact of individual studies in a sensitivity analysis. Potentially the authors could have considered using a proxy for person-years rather than length of follow-up."

We agree that it may have been possible to use a proxy for person-years, provided the individual studies reported the necessary information. However, given the time course for hepatocellular carcinoma to develop (i.e. up to a decade or longer), investigating length of follow-up as a source of heterogeneity was appropriate.

"Other minor issues and comments:
1) They use the Newcastle Ottawa Scale for the risk of bias assessment. I suggest, that the authors discuss the impact of this tool on the assessment results compared to the new ROBINS-I tool."

We acknowledge that there are many tools for assessing the risk of bias in non-randomised trials. These include, among others, the Newcastle Ottawa Scale, the ROBINS-I tool, the Downs and Black instrument and the ACROBAT-NRSI tool. The Newcastle Ottawa Scale is widely used and described in Chapter 13.5.2.3 of the Cochrane Handbook as one of “the most useful tools” and, thus, is an appropriate choice for our meta-analysis. While a discussion of the impact of the Newcastle Ottawa Scale in relation to the ROBINS-I tool (or any other tool) would be interesting, it would essentially amount to a critical appraisal of risk of bias assessment methodology, which is beyond the scope of our study.

"2) The search strategy seems simple, and more relevant synonyms could maybe provide more eligible studies. Please discuss."

We searched three large databases, some of which also included abstracts. We also manually searched the reference list of relevant articles found in our initial search. By way of comparison, our search returned all the studies found by previous meta-analyses in addition to other studies, which

highlights the ample coverage of our search. While we did not search foreign language databases, we have already explained the likely impact of this in the discussion.

"3) They excluded studies that ”did not report a dose-response or give sufficient information for calculation of a dose-response (i.e. this requires estimates for more than two exposure levels” and do not state if they contacted authors in order to try to retrieve missing data. If only a few were missing, contacting authors may have been appropriate. Please discuss."

As we had mentioned in the methods section, one study “reported RRs of HCC according to decaffeinated coffee consumption for three qualitative categories: “non-consumers”, “consumers below the median” and “consumers at/above the median”. We were unable to get the corresponding quantitative values from the authors so used those reported by another publication investigating“. This was the sole occasion we contacted study authors. We have amended the quoted passage and the “Searches and selection of studies” section to make this clearer.

"4) Their reference to the Cochrane handbook (heterogeneity), [22], should be changed into the primary references (i.e., Higgins, BMJ 2003;327:557) instead or the specific section/chapter."

We have amended the text and references to quote the specific chapter of the Cochrane Handbook.

"5) In the “Article summary”, the authors state “This is the first meta-analysis to calculate clinically relevant RRs of HCC for 1-5 cups of coffee per day”, however, the criteria used for deeming the effect clinical relevant is not stated in the text, and ‘clinically relevant’ is not mentioned further in the text. Please discuss."

In response to a previous comment from Reviewer 1, we have clarified the first sentence of the article summary by deleting the term “clinically relevant” and stating instead that our findings “may be useful in the design of a coffee based intervention for evaluation in a clinical trial”.

"6) No references are provided for the R packages (citation info is available online). Please include.
7) The cubic spline dose-response meta-analysis is several places called ‘cubic spine (…)’. Please remember ‘L’."

We thank Reviewer 4 for pointing this out. We have added references for the R packages, as suggested, and corrected “spine” to “spline”.

"8) The objectives are not stated in the main text. Furthermore, the objectives in the abstract and in the protocol are slightly ambiguous – you could get the impression, that caffeinated coffee will be compared to decaffeinated coffee. Please make it more clear to the reader."

We have amended the abstract to state: “Objective: To examine the association between coffee, including caffeinated and decaffeinated coffee, with hepatocellular carcinoma (HCC) and assess the influence of HCC aetiology and pre-existing liver disease”.

1. Bravi F, Tavani A, Bosetti C, et al. Coffee and the risk of hepatocellular carcinoma and chronic liver disease: a systematic review and meta-analysis of prospective studies. Eur J Cancer Prev 2016 doi: 10.1097/cej.0000000000000252.

VERSION 2 – REVIEW

REVIEWER	Sabrina Mai Nielsen Musculoskeletal Statistics Unit, The Parker Institute, Copenhagen University Hospital, Bispebjerg & Frederiksberg, Copenhagen, Denmark.
REVIEW RETURNED	17-Feb-2017

GENERAL COMMENTS	I accept the corrections and responses from the authors.